Impaired fibrinolytic activity in type II diabetes: Correlation with urinary albumin excretion and progression of renal disease

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Progression of renal disease and cardiovascular complications in type II diabetes mellitus have been shown to correlate with control of blood glucose, lipids, blood pressure, and smoking. These factors, however, do not appear to totally explain these diabetic complications. Renal disease and cardiovascular complications in type II diabetes are associated with vascular abnormalities and fibrosis, both of which may occur with impaired fibrinolysis. A cross-sectional study was therefore performed in 107 type II diabetic patients recruited from the Denver Metropolitan Area to examine the effect of impaired fibrinolysis, as assessed by the ratio of plasminogen activator inhibitor (PAI-1) to tissue-type plasminogen activator (t-PA). With urinary albumin excretion (UAE) as a risk factor for both renal disease progression and cardiovascular complications, the patients were analyzed with respect to UAE less than and greater than 1 gm/day. The age, blood glucose, hemoglobin A1C, duration of diabetes, lipids, body mass index, and smoking were no different between the groups. As expected, the group with greater UAE had worse renal function, the serum creatinine (1.98 ± 0.24 vs 1.21 ± 0.05 mg/dl, $P < 0.001$) and creatinine clearance (55.5 ± 6.0 vs 76.8 ± 2.7 ml/min, $P < 0.001$) were significantly different. The type II diabetic patients with greater UAE exhibited significantly higher PAI-1/t-PA (2.43 ± 0.26 vs 1.85 ± 0.07, $P < 0.03$). The past history of cardiac complications was also higher (87.5 vs 72.3%, $P < 0.07$) in the diabetic patients with more impaired fibrinolysis and greater UAE. Thus a prospective, randomized clinical trial in type II diabetes with PAI-1 inhibitors is needed.

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It is well established that vascular injury and cardiovascular diseases are the leading cause of death in diabetic patients[1,2] and diabetes is the major cause of end-stage renal disease.[3] Elevated urinary albumin excretion (UAE) is associated with increased risk of atherosclerotic cardiovascular mortality and progression of diabetic nephropathy in type II diabetes patients.[4–6] The pathophysiological mechanisms behind this observation are not clear. One of the critical responses to vascular injury is the activation of the plasminogen activation (PA) system including tissue-type (t-PA) and urinary-type plasminogen activator (u-PA), which convert plasminogen to plasmin. Plasmin in turn degrades fibrin and several extracellular matrix proteins. Fibrin deposition is an important factor in the development of vascular disorders such as atherosclerosis, and the accumulation of extracellular matrix proteins is a hallmark of renal fibrosis. By inhibition of t-PA and u-PA, as well as direct inhibition of plasmin, plasminogen activator inhibitor (PAI-1) may play an important role in the regulation of intravascular fibrin deposition, thrombosis, atherosclerosis, as well as renal fibrosis.[7–9] The resultant fibrosis and vascular injury in the kidney may be critical in the glomerular capillary leakage of proteins and resultant albuminuria. PAI-1 also can exert plasmin-independent effects on fibrin homeostasis by its capacity to react with other proteases such as activated protein C.[10] In addition to its role in the regulation of vascular fibrinolysis and fibrin deposition, PAI-1 exerts an effect on vascular smooth muscle proliferation.[7] The effects of PAI-1 on vascular pathology could be a possible link between albuminuria and cardiovascular diseases, as well as kidney disease progression in diabetic patients. The present study was therefore undertaken to examine the relationship between the ratio of PAI-1 and t-PA, as an index of fibrinolytic activity, UAE, and the progression of renal disease.

RESULTS

Characteristics of the study population

The clinical and biochemical characteristics of the total study population are shown in Table 1. The patients' average age was 65.2 years; and they had diabetes for a mean of 17.4

years. They were obese and mean creatinine clearance was 72 ml/min and UAE was increased. Their blood glucose control was not optimal as assessed by hemoglobin A1C.

A comparison of the patients with a UAE less than or equal to 1000 mg/day vs greater than 1000 mg/day are shown in Table 2. The patients were comparable with respect to age, blood glucose, hemoglobin A1C, duration of diabetes, plasma lipids, systolic and diastolic blood pressure, and smoking. As expected, the diabetic patients with UAE greater than 1 gm/day had significantly higher serum creatinine concentration and lower creatinine clearances (Figure 1a and b). With respect to impaired fibrinolysis in the diabetic patients, the PAI-1/tPA ratio was significantly higher in the patients with greater UAE (Figure 2). Over a 7-year mean follow-up, the rise in serum creatinine was larger in the diabetic patients with UAE >1000 (0.82 ± 0.21 mg/day) compared with patients with UAE $\leqslant 1000$ mg/day (0.07 ± 0.03, $P = 0.0002$).

Table 1 | Characteristics of the study population

N=107	Mean ± s.e.
Age (years)	65.18 ± 0.76
BMI (kg/m^2)	32.57 ± 0.53
Systolic BP (mm Hg)	142.70 ± 1.81
Diastolic BP (mm Hg)	80.87 ± 0.81
Glucose (mg/dl)	186.71 ± 6.59
Total cholesterol (mg/dl)	202.04 ± 4.19
TG (mg/dl)	219.08 ± 17.5
HDL (mg/dl)	40.37 ± 1.02
LDL (mg/dl)	120.43 ± 3.27
VLDL (mg/dl)	34.57 ± 1.85
HbA1C (%)	8.75 ± 0.14
Serum Cr (mg/dl)	1.39 ± 0.07
Cr clearance (ml/min)	71.99 ± 2.64
Duration of diabetes (years)	17.38 ± 0.76
PAI-1 (IU/ml)	21.19 ± 0.13
t-PA (IU/ml)	11.56 ± 0.83
UAE (μg/min)	628.12 ± 139.02

BMI, body mass index; BP, blood pressure; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HbA1C, glycosylated hemoglobin; Cr, creatinine; LVMI, left ventricular mass index; PAI-1, plasminogen activator inhibitor type 1; t-PA, =tissue-type plasminogen activator; UAE, urinary albumin excretion; s.e.=standard error.

DISCUSSION

Obesity and type II diabetes mellitus are increasing in epidemic proportions in the US.[11–13] Cardiovascular complications lead to approximately 50–70% of mortality in type II diabetes[1,2,14] and presently nearly 50% of new patients with end-stage renal disease are because of diabetes.[3] Results from prospective randomized studies have demonstrated that, in addition to control of blood glucose, aggressive antihypertensive therapy with a blood pressure (BP) goal of less than 130/80 mm Hg and inhibition of renin–angiotensin–aldosterone system decreases cardiovascular morbidity and mortality, stroke, diabetic retinopathy and diabetic nephropathy, and overall deaths.[15–18] Although risk surveillance of BP, hemoglobin A1C, lipids, and smoking is important in type II diabetic patients, an increased rate of UAE also has been shown to correlate with cardiovascular mortality[19,6] and progression of renal disease.

An increase in UAE indicates glomerular capillary albumin leakage, and also has been proposed to be an index of vascular damage throughout the body. While glomerular capillary leakage of albumin can be collected as urine, quantitation of interstitial albumin, as an index of capillary damage, is obviously not possible in the cardiovascular, retinal, and neurological systems. Thus, increased UAE has been suggested to reflect diffuse endothelial injury throughout the body.

The present study in 107 hypertensive type II diabetic patients examined another potential risk factor for cardiovascular complications and progression of renal disease, namely the activity of the fibrinolytic system. There is substantial evidence that thrombosis is an important pathogenic factor in myocardial infarctions and strokes. PAI-1 is important in modulating fibrinolysis and proteolysis. The conversion of plasminogen to plasmin with resultant fibrinolysis is stimulated by t-PA. Therefore, an increase in the PAI-1/t-PA ratio is as an index of impaired fibrinolysis.

We hypothesized that the impaired fibrinolysis in our type II diabetic patients would be significantly increased in those diabetic patients with greater UAE. This was indeed the case. Moreover, the patients with impaired fibrinolysis had diminished renal function and a greater loss of function over a 7-year follow-up period. The relationship between impaired fibrinolysis and proteolysis in those type II diabetic patients with the greater UAE was independent of any difference in age, blood glucose, duration of diabetes, blood lipids, systolic or diastolic blood pressure, and smoking.

Activation of the renin–angiotensin–aldosterone system in type II diabetic patients may also be involved in the inhibition of fibrinolysis. Angiotensin II can directly increase PAI-1 concentrations[20–22] and also indirectly stimulate PAI-1 level via an increase in TGF beta (TGF-β).[23] TGF-β, which is a potent profibrotic molecule, not only increases PAI-1 level but also induces extracellular matrix synthesis and fibrosis.[24] Furthermore, a high dose of an angiotensin II type 1 receptor antagonist attenuated glomerulosclerosis via a decrease in PAI-1 level.[25] As the patients in the present study were all receiving the angiotensin-converting inhibitor, enalapril, any effect of angiotensin II on PAI-1 and TGF-β would be attenuated, but probably not abolished.

The combination of decreased extracellular matrix degradation secondary to diminished fibrinolysis and proteolysis combined with increased extracellular matrix synthesis induced by TGF-β would be expected to increase tissue fibrosis. Cardiac fibrosis and renal interstitial fibrosis are hallmarks of diabetes-related cardiomyopathy and nephropathy, respectively. Thus, increased PAI-1/t-PA ratio may not only increase cardiovascular mortality by enhanced thrombosis but also by increasing cardiac fibrosis. The impaired fibrinolysis may also increase renal fibrosis and thus contribute to the progression of diabetic nephropathy. It has

Table 2 | Characteristics of patients with UAE ⩽1000 mg/day vs UAE >1000 mg/day

Variable	UAE ⩽1000 mg/day (*N*=83) Mean ± s.e.	UAE >1000 mg/day (*N*=24) Mean ± s.e.	*P*-value
PAI/t-PA (ng/ml)	1.85 ± 0.07	2.43 ± 0.26	0.0314
t-PA (ng/ml)	11.8 ± 0.56	10.6 ± 0.95	0.4818
PAI (ng/ml)	20.9 ± 0.99	22.3 ± 1.46	0.3283
HbA1c (%)	8.8 ± 0.16	8.6 ± 0.31	0.3492
Glucose (mg/dl)	183.9 ± 7.23	196.3 ± 15.56	0.5274
HDL (mg/dl)	40.3 ± 0.96	40.8 ± 3.14	0.3901
LDL (mg/dl)	118.4 ± 3.52	127.9 ± 8.10	0.2703
VLDL (mg/dl)	34.1 ± 2.12	36.2 ± 3.89	0.4739
Cholesterol (mg/dl)	199.2 ± 4.81	211.9 ± 8.39	0.1389
Triglycerides (mg/dl)	209.7 ± 18.99	251.1 ± 42.17	0.2298
Serum creatinine concentration (mg/dl)	1.2 ± 0.05	1.98 ± 0.24	0.0010
Creatinine clearance	76.8 ± 2.73	55.5 ± 5.96	0.0015
Average change in SCR over 6.8 years	0.09 ± 0.03	0.82 ± 0.21	0.000
Change rate in SCR (mg/dl/per/year)	0.01 ± 0.008	0.11 ± 0.035	0.0086
Weight (kg)	100.5 ± 2.20	89.5 ± 3.37	0.0109
Height (cm)	173.5 ± 1.14	172.5 ± 1.93	0.5366
BMI (kg/m^2)	33.3 ± 0.60	30.1 ± 0.98	0.0099
SBP (mm Hg)	141 ± 2.00	148 ± 4.01	0.2618
DBP (mm Hg)	81 ± 0.91	80 ± 1.80	0.8143
Diabetes duration (years)	17.1 ± 0.89	18.4 ± 1.50	0.2930
Age (years)	65.1 ± 0.86	65.3 ± 1.67	0.9851
Cardiovascular disease	60/83 (72.3%)	21/24 (87.5%)	0.0708
Smoking	52/83 (62.7%)	16/24 (66.7%)	0.7188

Note: Comparison of continuous variables was performed using Wilcoxon Sign-Rank test. Comparison of cardiovascular disease was performed using Fisher's Exact test. BMI, body mass index; SBP, blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HbA1C, glycosylated hemoglobin; PAI-1, plasminogen activator inhibitor type 1; t-PA, =tissue-type plasminogen activator; UAE, urinary albumin excretion; s.e.=standard error. Smoking defined as greater than 100 cigarettes.

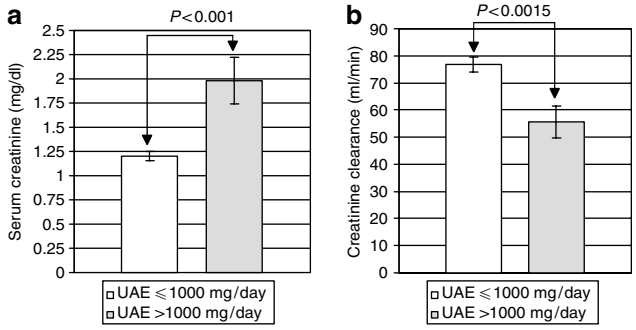

Figure 1 | Relationship between UAE and renal function.

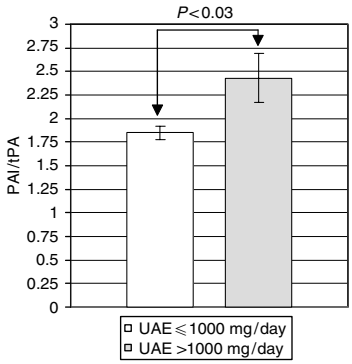

Figure 2 | Impaired fibrinolysis in type II diabetic patients with UAE greater than 1 gm/day.

been shown that diabetic mice null for PAI-1 (PAI-1$^{-}/^{-}$) showed less albuminuria and TGF-β expression than wild-type mice (PAI-1$^{+}/^{+}$).[26]

With respect to renal progression, there was a significantly higher serum creatinine concentration, as an index of kidney function in those patients with higher UAE and impaired fibrinolysis. Moreover, a retrospective evaluation also was undertaken to examine the incremental changes in serum creatinine concentration over a 7-year follow-up period in relationship to the fibrinolytic ratio. A larger increment in serum creatinine was observed in those patients with the greater UAE and higher fibrinolytic ratio. With impaired fibrinolyis, intravascular fibrin deposition may occur and contribute to intimal hyperplasia in the vasculature, a hallmark of diabetic cardiomyopathy and nephropathy. This vascular perturbation in diabetic patients also could increase vascular permeability and thereby contribute to the increased UAE. The endothelial dysfunction in these diabetic patients could be the common pathway for both the increased PAI-1 and UAE.

It should be recognized that there are several studies that have shown a correlation between UAE and PAI-1. These studies have been performed in healthy males with an average age of 58 years,[27] patients with essential hypertension[28] and type II diabetes.[29,30] In these publications, a relationship between impaired fibrinolysis and progression of renal disease has not been studied. No measurement of renal function (i.e. serum creatinine or creatinine clearance) was included in these papers[27–29] except in one of the publications.[30] In this study[30] however, creatinine clearance was reported in only diabetic patients with microalbuminuria and no correlation between UAE and PAI-1 was found. No diabetic patient with larger amounts of UAE was included in the study.

Lastly, it should be mentioned that a new potent inhibitor of fibrinolysis, the thrombin-activatable fibrinolysis inhibitor, has been identified and found to be higher in type II diabetic patients with microalbuminuria as compared to those diabetic patients with normoalbuminuria.[30] This inhibitor of fibrinolysis is in need of further study in diabetic patients with respect to cardiovascular disease and renal complications.

Thus, an impairment of fibrinolysis and proteolysis associated with a decrease in plasmin generation in hypertensive type II diabetic patients may predispose to thrombosis and fibrosis and thereby increase cardiovascular and renal morbidity and mortality. The numerous interactions that may occur in type II diabetic patients secondary to impaired fibrinolysis and proteolysis and thereby contribute to diabetic cardiomyopathy and nephropathy are shown in Figure 3. The results of the present study indicate that in addition to BP, smoking cessation, blood glucose and lipid control, impaired fibrinolysis, and proteolysis may be important contributors to diabetic cardiovascular and renal complications. A prospective randomized study is needed to test the hypothesis that inhibition of PAI-1 will diminish diabetic cardiovascular and renal complications.

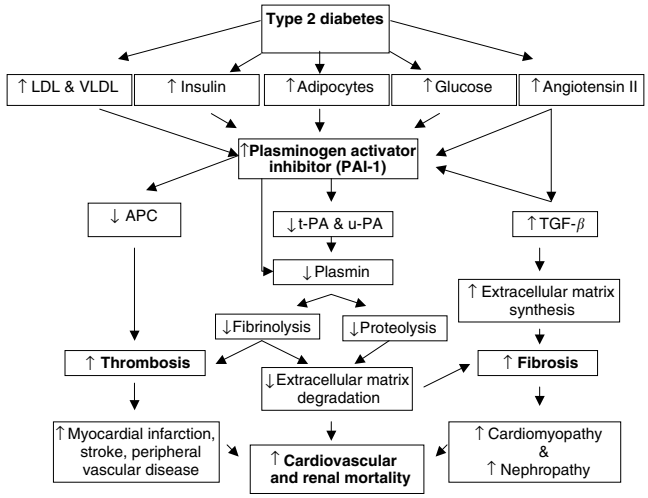

Figure 3 | Effects of PAI-1 in type II diabetes on cardiovascular and renal mortality: in type II diabetes, insulin, glucose, angiotensin II, obesity, and hyperlipidemia all increase PAI-1 level. TGF-β, which is stimulated by angiotensin II also increases PAI-1 secretion. Increased PAI-1 decreases plasmin level and thereby diminishes fibrinolysis and proteolysis. The resultant decrease in ECM degradation together with increased ECM production via TGF-β leads to ECM accumulation and fibrosis. In addition to its role in inhibition of fibrinolysis, PAI-1 also increases thrombosis by its interaction with other proteins such as APC. All the above events predispose to thrombosis and fibrosis and increased cardiovascular and renal morbidity and mortality in type II diabetic patients. APC = activated protein C, LDL = low density lipoprotein, VLDL = very low density lipoprotein, PAI-1 = plasminogen activator inibitor, t-PA = tissue-type plasminogen activator, u-PA = urokinase-type plasminogen activator, TGF-β = transforming growth factor β, ECM = extracellular matrix.

MATERIALS AND METHODS

From the hypertensive cohort of the Appropriate Blood Pressure Control Trial, 107 type II diabetic patients with albuminuria (UAE $>20\,\mu$g/min) residing in the greater Denver metropolitan region were enrolled in the present study to examine fibrinolytic activity. All patients were hypertensive (BP > 140/90 mm Hg) at the time of randomization for the Appropriate Blood Pressure Control Trial study to nisoldipine or enalapril. However, at the time of present study, all patients were on enalapril, because the patients on enalapril therapy were shown to have fewer myocardial infarctions during the first 4 years of the study than those patients on nisoldipine.[31] Patients in whom the assigned BP lowering goal was not achieved with the initial double-blind therapy received additional open-label antihypertensive therapy (atenolol and hydrochlorothiazide) as needed. Detailed description of patients' characteristics at the start of the Appropriate Blood Pressure Control Trial, including eligibility criteria, target and achieved BP, baseline kidney function, and kidney function during the 5-year Appropriate Blood Pressure Control Trial study have been published previously.[32,33]

Type II diabetes was diagnosed according to the criteria based on the World Health Organization report of 1985,[34] which followed the National Diabetes Group criteria of 1979.[35] Three BP measurements were taken at 2-min intervals in the sitting position. A standard mercury sphygmomanometer was used by trained nurses.

UAE was measured for individual patients on three separate occasions consisting of one 24-h urine collection and two overnight collections. The following formula was used to calculate the mean of UAE: albumin concentration (mg/dl) × urine volume (ml)/collection duration (min). UAE was categorized by stage: microalbuminuria (20–200 μg/min), overt albuminuria (>200 μg/min).

To calculate creatinine clearance values, patient's weight, urine, and serum creatinine were measured at the same time. The following formula was used to calculate creatinine clearance: creatinine clearance = 4.014 ((urine creatinine concentration × urine volume)/(serum creatinine concentration × collection duration × height$^{0.725}$ × weight$^{0.425}$)).

For PAI-1 and t-PA assays, blood samples were obtained at the Colorado Prevention Center and measured at the University of Texas Medical Center, Houston, Texas using standard methodology.[36] Because the balance between PAI-1 and t-PA is important in regulation of fibrinolytic system, PAI-1/t-PA ratio has been considered as an indicator of fibrinolytic system status. Other laboratory data were analyzed using a central laboratory: LabCorp, Denver, CO, USA. Fisher's exact test comparison of smoking was performed using χ^2 test of independence.

Statistical methods

Data were analyzed using SAS 9.1. Numerical data are presented as means and standard error of means. Comparisons in continuous variables were performed using non-parametric, Wilcoxon Sign-Rank tests. Presence of cardiovascular disease was tested using χ^2 test of Independence. *P*-values less than 0.05 were considered significant.

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